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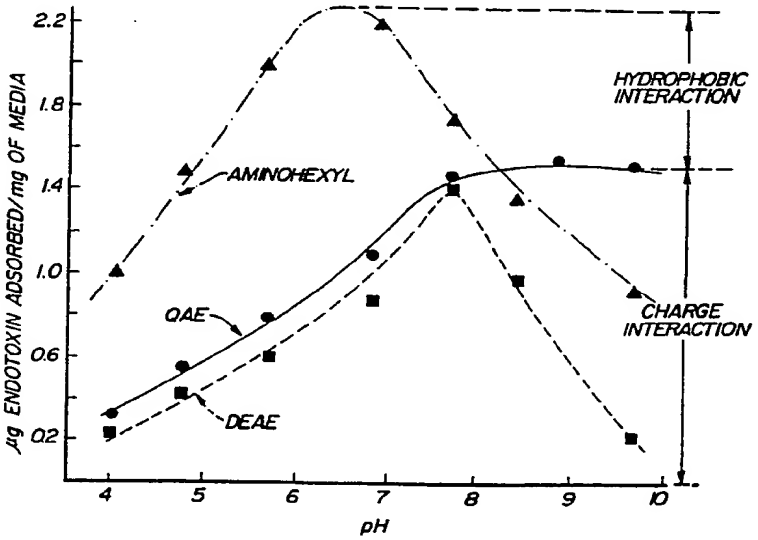
(54) Title: FILTER MEDIA AND USE FOR PYROGEN REMOVAL

(57) Abstract

A filter media is provided comprising a water insoluble carrier modified by a modifying polymer having a polymer chain and having along the polymer chain a pendent cationic substituent and a pendent hydrophobic substituent. Preferably the modifying polymer is made from a polymerization of: (a) a coumpound of the formula: (i) R^1 R^2 N--X--N R^3R^4, or (ii) R^1 R^2 N--X--N R^3--Y--N R^4R^5, wherein X and Y are each, independently, an aliphatic or aromatic substituent of 4 to 20 carbon atoms, and R^1, R^2, R^3, R^4 and R^5 are each, independently, a hydrogen or aliphatic substituent of 1 to 3 carbon atoms, and (b) a compound containing an epoxy group capable of direct coupling to an N on compound (a) and a vinyl group capable of bonding to the carrier. Preferably the media is used for removing pyrogen from aqueous compositions.

THE EFFECT OF pH ON ENDOTOXIN ADSORPTION



Endotoxin Conc.: 40µg E-coli 026:B6 endotoxin dispersed in 20ml of 0.1M buffer.
Media Adsorption: 25mg media dispersed in above solution and agitated for one (1) hour.
Assay: Quantitative Chromogenic 1000 LAL of Whittaker Bio-Products.

* See back of page

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Title: FILTER MEDIA AND USE FOR PYROGEN REMOVAL

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to a novel filter used for the reduction and removal of pyrogens from solutions, particularly aqueous or protein solutions. The novel filter of this invention provides for both hydrophobic and cationic charge forces for the capture of pyrogen. These forces provide a synergistic effect to enhance the capacity of the filter to adsorb pyrogen under a broad spectrum of conditions, particularly in the presence of salts. This is particularly useful for depyrogenating tissue culture media where salts are nutrient ingredients.

Prior Art:

Pyrogens are substances which abnormally raise the body temperature of an animal. When pyrogens are intermixed with blood in the human body, for example, by intravenous injection of a medicine contaminated therewith, the pyrogen causes severe fever. When the action of the pyrogen becomes serious, the fever is accompanied by chills and shudders and, occasionally, death from shock. Many substances, e.g. bacterial substances, inflammatory substances, vegetable polysaccharides, blood type substances are known as pyrogens.

Bacterial substances, e.g. bacterial toxins, are of greatest concern for they have the greatest influence on fever. Generally, bacterial toxins are classified as exotoxins or endotoxins. Endotoxins, the main component of which is cell wall-lipopolysaccharide (LPS) of gram negative bacterium, are the most pyrogenic. As used herein, the terms "LPS", "endotoxin", and "pyrogen" are considered synonymous.

Bacterial endotoxins have been recognized as a major cause of pyrogenic reactions during the administration of biological products. Control of endotoxins during the production of such products by strict aseptic techniques

that limit microbial contamination, in most cases, are ineffective due to the difficulty in maintaining complete sterility throughout the manufacturing process. Other known processes for depyrogenation may denature necessary protein molecules.

More specifically, pyrogens can be removed, for example, by: (1) adsorption by charcoal, ion exchange resins or the like, (2) decomposition with an acid or an alkali, (3) by oxidative decomposition with an oxidizing agent, such as potassium permanganate, aqueous hydrogen peroxide, sodium hypochlorite, and (4) filtration with an ultrafiltration membrane.

However, it is difficult to completely remove pyrogen by these known methods. Moreover, there are disadvantages in using such methods. For example, the use of adsorbents may result in the adsorption and loss of valuable product and the use of processes (2) and (3), above, may result in contamination and decomposition of the product.

More specifically, a number of methods have been reported for removing or reducing the level of endotoxins in fluids:

The chemical decomposition of pyrogens with acids, alkalis, and oxidizing agents is described for sterilizing liquids. (Pearson, F.C. III, Pyrogens, LAL Test and Depyrogenation, Marcel Dekker, N.Y.; 1985).

Dasinger, U.S. Patent No. 3,644,175, describes the inactivation (by acidification and heating) of endotoxin gram-negative bacteria intended for use as a protein source.

Smith, U.S. Patent No. 3,659,027, describes the destruction of pyrogens in water intended for parenteral use by strong alkali.

Akcasu, U.S. Patent No. 4,070,289, describes the depyrogenating of water by distillation under pressure.

Such harsh treatments are unsuitable for the purification of proteins.

Filtration using ultra membranes or depth type filters are also a means for removing pyrogen from biological solutions, see Gerba, C.P. and Hou, K.C., Appl. Environ. Microbiol 50, 1375-1377; 1985.

Pyrogens have a low isoelectric point due to the phospholipid groups thus making positively charged filter media useful for depyrogenating biological fluids, see for example, copending U.S. Serial No. 07/335,995 filed April 7, 19[illegible] entitled "Charge Modified Filter Media" to Ostreicher.

Carrazone, et al., "A New Type of Positively Charged Filter: Preliminary Test Results", Journal of Parenteral Science and Technology, 32:69-74, describes tests on Pall's ULTIPOR GF PLUS filters and states that such filters are effective in microbial removal but only when proteins or negative ions or peptones are not present in the solution. The filter media of the invention herein provides effective pyrogen removal in both strong electrolytes and proteinaceous solutions.

Robinson, et al., (1985), "Depyrogenation by Microporous Membrane Filters", in Technical Report No. 7, Depyrogenation, Parenteral Drug Association, Inc., Philadelphia, Pennsylvania; Mandaro (1987), "Charge Modified Depth Filters: Cationic-Charge Modified Nylon Membranes" in Meltzer (1987), "Filtration in the Pharmaceutical Industry", T.H. Meltzer Ed., Marcel Dekker, Inc., New York, New York, describe the limitations of

prior art cationic charge modified media in terms of general loss of filtration performance at high pH and, more specifically, in Robinson, et al. the inability of prior art media to achieve useful levels of very fine particle and/or pyrogen removal at high pH. The filter media of the invention herein exhibits useful filtration properties at high pH values.

Other relevant references:

GB No. 1,418,286 describes the removal of pyrogens from urokinase (a product of human urine) by retaining pyrogens on an anion exchange cellulose, such as diethyl-amino ethyl (DEAE) cellulose.

GB No. 1,557,545 describes reversibly adsorbing urokinase on a hydrophilic polysaccharide which does not retain pyrogens.

Chibata, U.S. Patent No. 4,381,239 reviews methods of removing pyrogen: (1) adsorption; (2) decomposition with acid or alkali; (3) decomposition with an oxidizing agent; or (4) filtration. Chibata further describes a method for removing pyrogen from a solution by contacting the solution with an adsorbent to adsorb the pyrogen. The adsorbent comprises a water-insoluble carrier and a

nitrogen-containing heterocyclic compound of the formula:

R——A——X

wherein R is a nitrogen-containing heterocyclic group; A is single bond, alkylene or alkenylene; X is hydrogen or a functional group; and the heterocyclic group and alkylene may be optionally substituted by one or more substituents, and the compound being bonded to the carrier directly or through a spacer. Cellulose is described as a preferred carrier. The process described in Chibata of producing the adsorbent is destructive of the carrier and the adsorbent produced has limited charge functionality.

U.S. Patents 4,663,163, 4,687,820 and 4,724,207 to Hou, et al. in their preferred embodiment describe polysaccharides, polypeptides and siliceous materials modified by a polymer of a reactive monomer such as glycidyl methacrylate (GMA) or glycidyl acrylate (GA), and another functional monomer such as diethylaminoethyl methacrylate (DEAE), or ß-carboxy ethyl acrylate (ß-CEA) to obtain an ion-exchange media for molecular separation or chromatography.

Hou, U.S. Patent Nos. 4,488,969 and 4,511,473 describe the incorporation of hydrophobic adsorbent such

as aerosil particles, in depth filters for pyrogen removal.

Olson, U.S. Patent No. 4,411,795 describes the removal of lipin particles, e.g. bacteria, yeast, fungi and viruses, from aqueous suspension by adsorption on hydrophilic macromolecules substituted with pendent hydrophobic groups. Preferred, are the use of pendent hydrophobes linked by ionogenic groups to insoluble carriers. Olson describes a process which only coats the carrier and does not provide for the selective removal of pyrogen.

Hao, U.S. Patent Nos. 4,677,194, 4,780,529 and 4,791,191, describes a method of isolating pyrogen inactivator from plasma for use as a bioligand for pyrogen inhibition. Affinity ligand coupling methods for pyrogen control are relatively expensive and require specific methods for coupling the ligand to a solid matrix.

Hou, 4,791,063, describes a polyionene-transformed modified polymer polysaccharide separation matrix having a relatively high molecular weight and low selectivity toward pyrogen removal.

Pyrogen adsorption by anion exchange resins and positively charged bio-ligands immobilized, for example, on sepharose can be attributed to the charge interaction mechanism. The negatively charged phosphate moiety of pyrogens is a functional group that interacts with positively charged matrices to enhance removal of pyrogen. A problem, however, is that the removal of pyrogen in the presence of protein molecules, such as albumin, by charge adsorption has always been difficult, especially where the protein molecules are also negatively charged.

Object and Summary of the Invention

It is an object of this invention to provide a novel filter media, particularly suitable for the filtration of biological or parenteral liquids to remove, inter alia, pyrogen.

A further object of this invention to provide a process for modifying filter elements to produce a filter media suitable for removing pyrogen.

Another object of this invention is to provide a filter media containing cellulosic fibers having a high capacity for the capture and adsorption of pyrogens, particularly at elevated pHs.

Yet another object of this invention is to provide a filter media capable of endotoxin, e.g. pyrogen, removal from fluids, particularly electrolytes or protein containing fluids.

A further object of the present invention is to provide a new and improved method of producing pyrogen-free water whch is readily adapted to large scale production.

Another object of the present invention is to provide a new and improved method of selectively depyrogenating protein containing fluids.

It is yet another object of this invention to provide a filter media which uses a combination of hydrophobic and cationic charge interaction forces for effecting a synergistic capture of pyrogen over a wide range of conditions.

It is still another object of this invention to provide a method of grafting onto a carrier a polymer chain of both hydrophobic and cationic-charged pendent groups such that the flexibility and number of functional groups surpasses conventional coating methods.

These and other objects of this invention are attained by a novel filter media comprising a water insoluble carrier modified by a modifying polymer having a polymer chain and having along the polymer chain a pendent cationic substituent and a pendent hydrophobic substituent. Preferably, the cationic substituent is selected from the group consisting of primary, secondary, tertiary and quaternary amino groups and the hydrophobic substituent is a C_4 to C_{20} alkyl or aromatic substituent.

More specifically, the filter media comprises a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) a compound of the formula:

(i) R^1 R^2 N--X--N R^3R^4, or

(ii) R^1 R^2 N--X--N R^3--Y--N R^4R^5

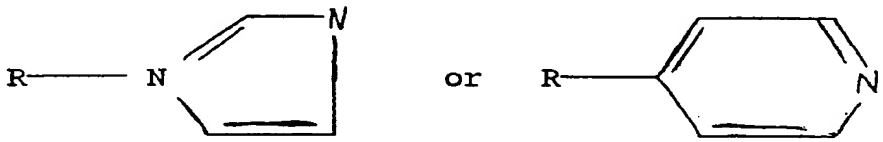
wherein X and Y are each, independently, an aliphatic or aromatic substituent of 4 to 20 carbon atoms, and

R^1, R^2, R^3, R^4 and R^5 are each, independently, a hydrogen or an aliphatic substituent of 1 to 3 carbon atoms, and

(b) a compound containing an epoxy group capable of direct coupling to N on compound (a) and a vinyl group capable of bonding to the carrier.

Another embodiment of the filter media comprises a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) a heterocylic nitrogen compound having the formula:



wherein R is an alpha, beta-ethylenically unsaturated polymerizable radical, and

(b) a compound containing an epoxy group capable of direct covalent coupling to a substituent on the carrier and a vinyl group capable of free radical polymerization to R on compound (a).

This invention is further directed to a process for modifying the water insoluble carrier by applying to the carrier the aforesaid modifying polymer. The process for modifying the carrier may comprise contacting the substrate with (i) a solution of the modifying polymer or (ii) solutions of compounds (a) and (b), either sequentially or simultaneously, to form the polymer in situ, and then curing the carrier to react compounds (a) and (b) to form the polymer and to bond the polymer to the carrier surfaces. The filter media of this invention may be used for the filtration of fluids, particularly parenteral or biological liquids containing proteins, to remove pyrogens.

There is also provided herein a novel method for reducing the pyrogen content of pyrogen-containing solutions. This is accomplished by contacting the pyrogen-containing solution with the aforesaid media,

preferably by passing it through the media.

The preferred carriers are cellulose, nylon or silica, with cellulose the highly preferred carrier.

It is theorized that the cationically charged substituents interact with the negatively charged phosphate ester groups in the pyrogen to assist in the removal of pyrogen. If, however, salt is present, it will mask the positive charge sites on the carrier inhibiting pyrogen interaction. The presence of protein will also inhibit the interaction of pyrogen with the charge sites on the filter. Under such conditions, the hydrophobic groups on the carrier assist in the removal of pyrogen. Together, the cationic groups and hyrophobic groups interact to synergistically capture and remove pyrogen from pyrogen-containing solutions under a broad spectrum of conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the contributions of hydrophobic and cationic charge forces to pyrogen adsorption. Primary amine groups have the weakest positive charge and thus demonstrate maximum charge or capacity at an acidic pH of 6-7. Tertiary amine groups, e.g. DEAE, have a maximum charge or capacity at about pH 8, quaternary amine groups, e.g. QAE, have a maximum charge or capacity at pH 10. The contribution to pyrogen adsorption capacity of hydrophobic groups, particularly due to the C_6 alkyl groups of aminohexyl substituents (See Example 1), accounts for about a 40% increase in capacity for pyrogen adsorption.

Figure 2 is a graph showing the effect of the addition of a C_6 or C_{10} hydrophobic alkyl substituent to the carrier. This hydrophobic substituent compensates for the loss of pyrogen adsorption by the filter due to increasing concentrations of salt which inhibit charge interaction.

Figure 3 shows that the hydrophobic substituent, a C_6 alkyl group, enhances pyrogen adsorption of the filter media at high concentrations of salt.

Figure 4 shows:

(a) typical pyrogen structure;

(b) charge modified media of prior art; and

(c) the media of this invention.

Figure 5 is a hypothetical mechanism depicting the hydrophobic and charge interaction between pyrogen and the filter media of this invention.

Figure 6 shows the pH range for removal of pyrogen from albumin by filters made according to Example 1 (decylamine).

Figure 7 shows the pH range for removal of pyrogen from gamma globulin by filters made according to Example 1 (decylamine).

Figures 8-12 depict embodiments of this invention wherein both hydrophobic and charged groups are bonded to a filter substrate.

Figure 13 is discussed in Example 6.

Figure 14 is discussed in Example 5.

DETAILED DESCRIPTION OF THE INVENTION

The water-insoluble carrier may be any water-insoluble carrier wherein the cationic and/or hydrophobic substituents can be bonded, either directly or indirectly through a spacer. Preferably the carrier is hydrophilic.

By the use of the term "hydrophilic" in describing the carrier, it is meant a carrier which adsorbs or absorbs water. Generally, such hydrophilicity is produced by a sufficient amount of hydroxyl (OH-), carboxyl (-COOH), amino (NH_2), halogen and/or similar functional groups on the surface of the substrate which assist in the adsorption and/or absorption of water into the substrate. Such functional groups are highly desirable in providing the adequate bonding of the modifying polymer to the substrate.

The preferred carrier is a polysaccharide. The term "polysaccharide" as used in the specification and claims is meant to include compounds made up of many -- hundreds or even thousands -- of monosaccharide units per molecule. These units are held together by glycoside linkages. Their molecular weights are normally higher than about 5,000 and up into the millions of daltons.

They are normally naturally occurring polymers, such as, for example, starch, glycogen, cellulose, gum arabic, agar, and chitin. The polysaccharide should have one or more reactive hydroxy groups. It may be straight or branched chain.

The preferred polysaccharide for the purposes of this invention is cellulose. "Cellulose" is intended to mean any of the convenient and commercially available forms of cellulose, such as wood pulp, cotton, hemp, ramie, or regenerated forms such as rayon. There exists no criticality as to the selection of a suitable form of cellulose. Cellulose is a naturally occurring polysaccharide consisting of beta -1,4 linked glucose units. In the native state, adjacent cellulose chains are extensively hydrogen bonded forming microcrystalline regions. These regions are interspersed by amorphous regions with less hydrogen-bonding. Limited acid hydrolysis results in preferential loss of the amorphous regions and gives so-called microcrystalline cellulose. The cellulose useful in the present invention is either cellulose in the native state, or in the microcrystalline state. Also, cellulose derived from cotton linter is preferable to that derived from wood pulp, as the latter contains lignin.

Preferred examples of a water-insoluble carrier having hydroxyl substituents are a polysaccharide (e.g. cellulose, agarose, cross-linked dextran, etc.). Other carriers contemplated are nylon, e.g. nylon 66 microporous membrane, and silica. The preferred media comprises a polysaccharide carrier modified by an organic synthetic polymer.

More specifically, the filter media comprises a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) a compound of the formula:

(i) R^1 R^2 N--X--N R^3R^4, or

(ii) R^1 R^2 N--X--N R^3--Y--N R^4R^5

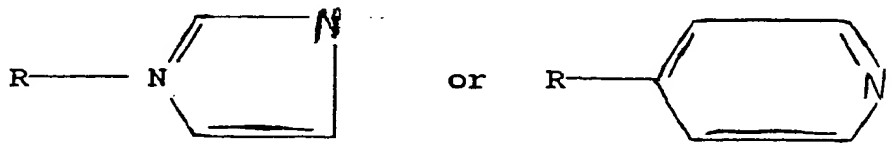
wherein X and Y are each, independently, an aliphatic or aromatic substituent of 4 to 20 carbon atoms, (preferably 6 to 12 carbon atoms), and

R^1, R^2, R^3, R^4 and R^5 are each, independently, a hydrogen or an aliphatic substituent of 1 to 3 carbon atoms (preferably hydrogen), and

(b) a compound containing an epoxy group capable of direct coupling to N on compound (a) and a vinyl group capable of bonding to the carrier (preferably glycidyl methacrylate).

Another embodiment of the filter media comprises a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) a heterocylic nitrogen compound having the formula:



wherein R is an alpha, beta-ethylenically unsaturated polymerizable radical (preferably vinyl imidazole vinyl, pyridine), and

(b) a compound containing an epoxy group capable of direct covalent coupling to a substituent on the carrier and a vinyl group capable of free radical polymerization to R on compound (a), e.g., glycidyl methacrylate.

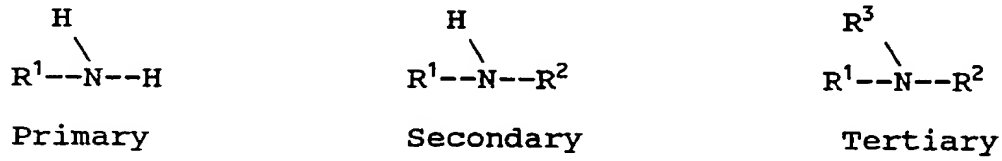
Comonomer (b), above, preferably contains vinyl unsaturation to promote polymerization and/or copolymerization with other monomers and/or the carrier and, at the same time, contains a group capable of covalently bonding to the carrier and/or other monomers through the hydroxyl, carboxyl, halogen and amino substituents thereon. Preferred groups include glycidyl

groups. Among the compounds containing a glycidyl group are the ethers or esters formed between a glycidyl alcohol and an unsaturated alcohol or unsaturated caboxylic acid. Typical glycidyl alcohols are aliphatic and cyclo-aliphatic alcohols and other alcohols having from 3 to 18 carbon atoms which are esterified with an alpha, beta-unsaturated carboxylic acid, preferably acrylic or methacrylic acid, or are etherified with olefinically or acetylenically unsaturated alcohol. Preferred compounds are glycidyl acrylate (GA) and glycidyl methacrylate (GMA). Other comonomers may be 4-5-epoxy-pentyl acrylate; 4-(2,3-epoxy propyl)-N-butyl methacrylate; 9,10-epoxystearyl acrylate; 4-(2,3-epoxy propyl)-cyclohexyl methacrylate; ethylene glycol-monoglycidylether acrylate, and allyl glycidyl ether and the like.

Comonomer (a) is a polymerizable compound carrying both cationic and hydrophobic chemical groups or substituents. The cationic substituent is selected from the group consisting of primary, secondary, tertiary and quaternary amino groups.

Amines are classified as primary, secondary or tertiary, according to the number of substituents

attached to the nitrogen atom, i.e., according to the number of hydrogens which have been substituted:



Epoxide groups from compound (b) will react with primary and secondary amine groups through the free hydrogens. An epoxide group will not react with a tertiary amine group since there are no free hydrogens.

Preferred among comonomers (a) are alkyl diamines, e.g., hexamethylene diamine; amino alkyl oligomers, e.g., bis(hexamethylene) triamine; aromatic diamines, e.g. diamino phenyl amino, diamino diphenyl amino and other comonomers such as vinyl imidazole, N(3-amino propyl methacrylamide).

The modifying polymer should have a sufficient amount of comonomer (b) to permit substantial coupling of the modifying polymer to the carrier. If too little comonomer (b) is present in the polymer, then grafting becomes difficult, if not impossible. Generally, about 4% to 20% by weight, preferably 5% to 10% by weight of

comonomer (b) relative to the total of (a) plus (b) is needed.

The free radical addition polymerization of free radical polymerizable comonomers (a) and (b) is carried out with free radical initiators using the steps of initiation, addition and termination. Such procedures are well known in the art. A preferred procedure is to utilize a substance or substances which produce radicals capable of reacting with the monomers. Probably the simplest of all polymerization initiators are the organic peroxides and azo compounds. These substances decompose spontaneously into free radicals in common organic solvents at a finite rate, at temperatures between 50 and 140°C. For example, benzoyl peroxide decomposes into two benzoyloxy radicals at 60°C. Another example is afforded by the azo compound azo-bis-isobutyronitrile (AIBN) which similarly decomposes into radicals at easily accessible temperatures.

The necessary energy may also be provided by irradiating the initiator system with ultraviolet light. For example, initiation can be provided by irradiating the initiator system in the presence of photo initiators such as benzophenone and its derivatives, benzoin alkyl

ethers or derivatives, or acetophenone, with ultraviolet light. It is then necessary that the initiator molecules absorb in the spectral region supplied. In this way radicals can be generated at a finite rate at considerably lower temperatures than are necessary if purely thermal excitation is used. Finally, bimolecular reactions may produce radicals capable of initiating polymerization. Particularly important are the redox reactions, which occur in aqueous media, and involve electron transfer processes. For example, the system Fe(II) plus hydrogen peroxide, or Ag(I), plus S_2O_8 -- are particularly important in initiating the radical polymerization of monomers. Because of the low temperature of initiation, the redox initiators or photochemically induced initiators are particularly preferred in the present invention. The amount of initiator is that sufficient to initiate the polymerization reaction. Polymerization is carried out until substantially all of the monomers or comonomers have been incorporated into the polymeric chains. This can be readily ascertained by simple analytical tests on the reaction mixture. Preferably, this polymerization is accomplished just prior to the covalent coupling of the polymer to the carrier. Preferably, the coupling and polymerization are performed in the same liquid phase.

The most preferred method of carrying out the process is in a "one-pot" system. All desired comonomers are added to an inert solvent system, such as, e.g., water, alcohols, organics, and the like, preferably producing a clear coating solution of the modifying polymer. The preferred solvent is water. The comonomers are treated under conditions which will initiate polymerization of the comonomers. This can be accomplished, for example, by adding to a well stirred mixture a water solution of an initiator, e.g. ammonium persulfate (APS), sodium thiosulfate (STS), and initiating polymerization at from about 60°C. to 100°C. Alternatively, a photolabile initiator can be added and initiation caused by photochemical means. After stirring for a time sufficient to allow the polymerization to proceed to completion, the linking of the formed copolymer to the carrier is caused by applying the modifying polymer to the carrier causing condensation of the modifying polymer to the carrier.

In the case when the linking group on the copolymer is a glycidyl group, it may be desirable to heat the polymer to cause such condensation; such temperature is normally around 80°-100°C. Reaction time is then allowed to proceed for a time sufficient to either go to

completion, or to achieve modification of the carrier to the desired capacity. The product is then washed and dried for further treatment, if necessary.

The amount of modifying polymer used is an amount sufficient to enhance the capture of pyrogen material on the membrane. Such an amount is highly dependent on a specific modifying polymer and carrier utilized.

The weight ratio of carrier to the modifying polymer bonded thereto is freely adjustable, and varies from 1.0% to 200%, by weight, of polymer to carrier. The preferred weight ratio of modifying polymer bound to the carrier is in the range of about 10% to 50%.

Broadly, the process of this invention is directed to modifying a water insoluble carrier, which is preferably hydrophilic, to enhance the capture potential of the carrier for pyrogenic material. The process comprises applying to the carrier a modifying amount of the aforesaid modifying polymer.

Although Applicants do not wish to be bound by the following theory, it is believed that in bonding the modifying polymer to the carrier the epoxide groups on

the polymer enter into addition type reactions with the hydroxyl, carboxyl and primary and secondary amines, which are on the hydrophilic carrier or on the other comonomer.

The modifying polymer is adsorbed onto the carrier elements and bonded to substantially all of the wetted surfaces of the carrier elements, i.e., to substantially all of the microporous microstructure of a subsequently formed filter media.

By the use of the term "bonded" it is meant that the modifying polymer is sufficiently attached through covalent bonding to the carrier or filter elements and/or to each other so that they will not significantly extract from the filter media under the intended conditions of use. By the use of the term "substantially all of the wetted surface" as used herein it is meant substantially all of the external surface and internal pore surfaces which are wetted by a fluid passing through the filter media or in which the media is immersed, i.e., substantially all of the microporous microstructure of the filter media.

A preferred filter media of the present invention is a filter media sheet comprised of filter elements of

silica based particulate immobilized in a porous matrix of cellulose fibers, both of which are modified by the modifying polymer. The preferred cellulose fibers are derived from wood pulp. Optionally, cellulose fibers, wherein the cellulose is highly purified alpha-cellulose, provide a filter media, which eliminates false positive tests for pyrogen and is capable of producing filtrates demonstrating very low levels of pyrogen, as tested by the LAL pyrogen test. See U.S. Patent No. 4,606,824 to Chu, et al incorporated herein by reference.

In the preferred embodiment, in order to provide a matrix which is a coherent and handleable sheet for use, it is desirable that at least one of the components that goes into forming the porous matrix is a long self-binding structural fiber. Such fiber gives the filter sheet media sufficient structural integrity in both the wet "as formed" condition and in the final dried condition. Such a structure permits handling of the filter media during processing and at the time of its intended use. Such fibers are particularly suitable in diameters in the range of 6 to 60 micrometers. Wood pulp, for example, has fiber diameters ranging from 15 to 25 micrometers, and fiber lengths of about 0.85 to about 6.5 mm.

When the amount of particulate immobilized in the porous matrix is low, i.e. less than about 50% by weight of the media, it is preferred that the porous matrix be formed of a self-bonding matrix of normal cellulose pulp having a Canadian Standard Freeness (CSF) of +400 to +800 ml. The state of refinement of wood pulp fibers is determined by means of a "freeness" test in which measurement of the flow rate through the fibers on a standard screen is determined. Two of the most common instruments are the "Canadian Standard Freeness Tester" and the "Shopper-Riegler Freeness Tester". For a more detailed explanation of these tests, see U.S. Pat. No. 4,309,247 to Hou, et al., the entire disclosure of which is incorporated herein by reference. Typical wood pulps show Canadian Standard Freeness values ranging from +400 to +800 ml.

In a preferred embodiment of this invention it is desirable to have a high amount, i.e. greater than about 50% by weight of the filter media, of particulate immobilized in the porous matrix, the remainder being cellulose fiber filter elements. It is thus highly desirable to use the invention described in the aforementioned U.S. Pat. No. 4,309,247 to Hou, et al to maintain such high content of particulate in the filter

media. Broadly, a portion of cellulose pulp refined to a Canadian Standard Freeness of between about +100 and -600 ml is incorporated with a portion of the normally dimensioned cellulose pulp (+400 to +800 ml). Generally the weight ratio of unrefined to highly refined pulp will range from about 0.1:1 to about 10:1, preferably 0.2:1 to about 1:1. Such a mixture of pulps permits the retention of fine particulates up to about 80% by weight of the filter media. The higher ratios produce media which are more porous. In any event, it is essential that the cellulose, both refined and unrefined, be a highly pure cellulose. Thus the entire cellulose content of the filtration media comprises a highly pure cellulose, the cellulose with a Canadian Standard Freeness of +400 to +800 ml and the cellulose with a Canadian Standard Freeness of -100 to -600 ml each being highly pure.

Preferably the filter media, and in particular the filter media sheet, is formed by vacuum-felting an aqueous slurry of such normal cellulose fibers, highly refined wood pulp, and particulate with the modifying polymer. This forms a modified filter media sheet having the particulate immobilized in a porous matrix. The final dried and cured filter media sheet shows a uniformly high porosity and a fine pore-sized structure

with excellent filtration flow characteristics.

The amount of particulate in the filter media may be as little as 20% by weight of the filter media up to about 80% by weight. Generally, levels of about 50 to 70% by weight are employed. Various types of siliceous particulate are suitable for inclusion in the filter media of this invention, including diatomaceous earth, perlite, talc, silica gel, clay, etc. In a broad sense, any fine particulate may be suitable, such as J.M. Filter Cel, Standard Super Cel, Celite 512, Hydro Super Cel, Speed Plus and Speed Flow; Dicalite 215 and Dicalite 416 and Dicalite 436. Siliceous fibers, e.g., glass fibers, may also be used either alone or admixed with the particulate.

In one embodiment herein, at least some of the particulate material may be "micro-particulate", i.e., has on the average a diameter of less than one micron, (a Gaussian distribution of particle diameters), preferably less than 100 millimicrons, most preferred less than 50 millimicrons, especially between 1 and 25 millimicrons. The micro-particulate is preferably fumed silica or fumed alumina; see U.S. Pat. No. 4,511,473 to Hou, et al, the entire disclosure of which is incorporated herein by reference.

In another embodiment, the cellulose-containing separation media contains, as at least a portion of the particulate, activated carbon particles. The carbon particles have an average diameter of less than about 50 microns; see U.S. Patent No. 4,404,285 to Hou, the entire disclosure of which is incorporated herein by reference.

In still another embodiment, the modified carrier may have a polyionene bonded thereto, see Fig. 13, and see U.S. Patent No. 4,791,063 to Hou et al, the entire disclosure of which is incorporated herein by reference.

The sequence of adding the required components to water to form the dispersed slurry of filter elements and modifying polymer appears to be relatively unimportant provided that the slurry is subjected to hydrodynamic shear forces during the mixing process. Preferably, the modifying polymer or components are added last. Preferably, refined pulp is added to a slurry of unrefined pulp and then the particulate incorporated in the slurry. The slurry is normally prepared at about 4% consistency, i.e., weight percent solids, and then diluted with additional water to the proper consistency required for vacuum-felting sheet formation. This latter consistency value will vary depending upon the type of

equipment used to form the sheet.

The process conditions are not critical as long as the modifying polymer or components thereof are permitted to contact the filter or carrier elements contained in the slurry. The amount of the dispersion medium, e.g. water, does not appear to be critical. The time required for modification of the surface and adsorption into the filter elements does not appear critical and appears to occur within about 0.5 to about 5 hours being adequate for most purposes. Of course, longer periods of exposure can be used to assure relatively complete adsorption, reaction, bonding and deposition of the modifying polymer. A period of about 1 to 3 hours is typical.

The amount of modifying polymer added to the filter material is not critical but is merely a matter of functionality. For example, a high surface area filter elements may require more modifying polymer for optimum filtration than one of lower surface area. Nevertheless as the polymer is adsorbed into the filter elements and deposited and bonded on the surfaces thereof, the filtration efficiency is enhanced, so that even small amounts are effective.

The filter media of this invention are free of extractables and free of discoloration, such that the sheets are usable under any sterilizing conditions and may be employed safely and effectively with potables or ingestables such as food or drugs. Additionally, such filter media has an unexpectedly high capability for removing pyrogen from fluids, particularly electrolytes and proteinaceous solutions, as well as maintaining filtration effectiveness at high pH's, e.g., up to about 12.

A preferred form of utilizing the filter media of this invention is to incorporate the filter media in sheet form in a filter cell which is used to form a filter cartridge. Such filter cartridges are of the type sold by Cuno, Incorporated (Meriden, Connecticut) under the trademark ZETA PLUS. Several embodiments of this form of filter cell and cartridges are described in U.S. Patent No. 4,347,208 to K. Southall; No. 4,783,262 to Ostreicher, et al; 4,606,824 to Chu, et al; and 4,704,207 to Chu. The entire disclosures of these patents are incorporated herein by reference.

Another form of utilizing the filter media of this invention is to incorporate the filter media in

cartridges similar to that described in U.S. Patent Nos. 4,675,104 to Rai, et al and 4,791,063 to Hou, et al.

EXAMPLES

Methods

Endotoxin of chromatographically purified escherichia coli 0111:B4 lipopolysaccharide (Sigma Chemical Co., St. Louis, MO) was used in all challenge studies. Pyrogen free water (American McGraw, Irving, CA) was used for the dilution of endotoxin, protein determination and buffer preparation.

The chromogenic Limulus Amebocyte Lysate (LAL) test kit from Whittaker, M.A. Bioproducts was used to determine endotoxin concentration.

In the chromogenic LAL test, the proenzyme is activated by the endotoxin in a water bath at 37°C for 10 minutes. The reaction is stopped with 50% acetic acid. The active enzyme then causes the release of P-nitroaniline from the substrate, producing a yellow color. The intensity of the color change produced by the substrate cleavage is measured on the Dynatech 96 Microplate Reader, Model MR 60, at 405 mn photometrically. The correlation between the absorbance and the endotoxin concentration is linear in the 10 pg to 0.1 ng/ml range.

Bovine Serum Albumin (BSA) (Sigma, Corp.) was prepared from fraction V powder and contaminated with 10 ng/mg of pyrogen contamination as measured by the chromogenic LAL test.

Gamma globulin purified from Cohn fraction II & III (Sigma, Corp.) was found to be pyrogen free. The protein solution was prepared by using pyrogen free water and the concentration was measured at 280 nm spectrophotometrically.

All the glass wares were depyrogenated at 180°C for 8 - 12 hours and the tubings and cartridges were depyrogenated by flushing with 3.0% hydrogen peroxide solution for 30 minutes to ensure the whole system is pyrogen free.

Static Test Method

Test tube experiments were performed by dispersing endotoxin in buffer solutions with weighed amounts of media in sterile tubes at room temperature, then agitating for 1 hour in a shaker. The media was then spun down, and the supernatants assayed to measure the reduction of endotoxin concentration. Each test tube contained 10 μg of E.coli endotoxin dispersed in 5 ml or 20 mM buffer mixed with 10 mg of media. The fibrous

media gradually swelled and became uniformly dispersed in the buffer during agitation. Sodium acetate buffer was used at pH below 7, whereas sodium phosphate buffer was used at a pH above 7.

Dynamic Flow Test

The dynamic flow test was performed by pumping 3.5 liters of buffer solution containing 20 nano-grams pyrogen/ml and 2.0 mg/ml of BSA through a 250 ml nominal size cartridge. The cartridge contained 40 grams of test media, and was pre-flushed with 1 liter of 3% H_2O_2 solution as a depyrogenating procedure followed by equilibration with 0.1 M Tris buffer at pH 8.5. Samples were collected every 200 ml for analysis of BSA and pyrogen concentrations at the flow rate of 40 ml/min.

EXAMPLE 1

MEDIA PREPARATION

(GMA/DIAMINO ALKYL)

(a) Formulation

Reagent	Quantity
Refined cellulose	5.0 g
Glycidyl methacrylate (GMA)	12.5 ml
Ammonium persulfate (APS)	0.5 g
Sodium thio-sulfate (STS)	0.5 g
D.I. water	250.0 ml
Diamino alkyl	20.0 ml

(b) General Process of Manufacture

The cellulose was dispersed in deionized (D.I.) water with agitation and heated to 80°C, with agitation. The glycidyl methacrylate, APS and STS, were added to the reactor and the reaction permitted to proceed for one hour. Then the diamino alkyl was added and the reaction permitted to proceed for an additional 1 to 3 hours. The diamino alkyl compounds are of the type $NH_2(CH_2)_nNH_2$ with n ranging from 4 to 20, preferably between 6 to 12 (See Fig. 8). The reaction was terminated. The media matrix was then washed with 5 x 1.8 liters of D.I. water and stored for further processing.

c) Use

Fifty (50) mg of the media produced by the above procedure was mixed with 20 mls of a 50 mM buffer (adjusted to different p^H's)

containing 1 μg/ml E.coli pyrogen, at room temperature for 15 minutes.

The amount of pyrogen removed by the media was assayed by the Whitaker Chromogenic LAL test previously described.

Experiments with varying hydrophobic alkyl groups, pH, and salt concentrations are shown in Tables 1 and 2.

TABLE 1

PYROGEN REMOVAL AT VARYING LENGTH OF HYDROPHOBIC ALKYL GROUP AND VARYING pH

		E-coli pyrogen removed (µg/gram media)		
		n=1	n=6	n=10
Buffer Solution	pH	mono-amine	Hexylamine	Decylamine
Sodium acetate	5.0	27	225	232
	5.7	29	261	272
Sodium phosphate	6.4	66	319	543
	7.1	62	329	617
	7.8	55	318	672
Sodium borate	8.5	42	282	710
	9.25	28	223	513

Also see Fig. 1.

TABLE 2

PYROGEN REMOVAL AT VARYING CONCENTRATION AND VARYING LENGTH OF HYDROPHOBIC ALKYL GROUP

Salt Concentration	Amount of pyrogen removed					
	n=1 mono amine		n=6 Hexylamine		n=10 Decylamine	
0	213 µg/g	100%	411 µg/g	100%	803 µg/g	100%
0.05 M	211 µg/g	99%	456 µg/g	111%	807 µg/g	100%
0.10 M	206 µg/g	97%	475 µg/g	115%	782 µg/g	97%
0.20 M	186 µg/g	87%	453 µg/g	110%	778 µg/g	97%
0.40 M	84 µg/g	39%	401 µg/g	97%	778 µg/g	97%
0.70 M	59 µg/g	27%	256 µg/g	62%	756 µg/g	94%
1.00 M	17 µg/g	8%	120 µg/g	29%	743 µg/g	92%

EXAMPLES 2 AND 3

COUPLING OF AMINO ALKYL OLIGOMERS AS PYROGEN ADSORPTIVE GROUPS TO THE MATRIX

The oligomers having the general structure of $H_2N(CH_2)_nNH_2(CH_2)_mNH_2$, both n and m are ranging from 4 to 20, with 6 to 12 preferred. Bis (hexamethylene) triamine (BHMT) is a preferred example $H_2N(CH_2)_6NH(CH_2)_6NH_2$.

(a) Formulation:

D.I. water	230 ml
(LAE)	0.40 g
+120 pulp	5.0 g
Glycidyl methacrylate (GMA)	12.0 ml
Ammonium persulfate (APS)	0.50 g in 10 ml H_2O
Sodium thiosulfate (STS)	0.50 g in 10 ml H_2O
NaCl	10.0 g
Bis (hexamethylene) triamine (BHMT)	19.0 g

(b) Procedure for Manufacturing

1. Water, LAE, and cellulose were mixed together, stirred at 300 rpm for 30 minutes while purging with N_2.

2. GMA was added and dispersed therein at high speed for 2 minutes.

3. The initiator solutions were added (APS first, and then STS) and the reaction flasks heated as quickly as possible to 80°C. (Heat-up time about 10 minutes.) The reaction was permitted to proceed at this temperature for 1 hour.

4. NaCl and BHMT were then added and the reaction continued for 4 additional hours at 80°C, and about 1000 rpm.

5. The reaction product was quenched and washed 5 times with 3.5 liter portions of D.I. water. See Figure 9.

(c) Use

One (1) gram of the resultant media was packed into a 16 mm diameter column and equilibrated with 20 ml of 100 mM sodium phosphate buffer at pH 6.5. 200 ml of a 10 ng/ml pyrogen solution were passed through the packed column at 2 ml/min. Fractions of each 50 ml were collected and assayed for pyrogen removal. Table 3 shows the results of these tests.

TABLE 3

PYROGEN REMOVAL BY COLUMN PACKED WITH MEDIA

Example No.	Volume collected (ml)	E.coli pyrogen concentration in solution (ng/ml) Before	After	% Removal
2	50	10	0.24 ml	
	100	10	0.31	
	150	10	0.44	
	200	10	0.41	
	Total	2000 ng	0.33	96.7
3	50	10	0.92	
	100	10	0.46	
	150	10	0.83	
	200	10	0.82	
	Total	2000 ng	0.82	91.8

EXAMPLE 4

FORMATION OF COPOLYMERS CARRYING IMIDAZOLE GROUPS AS PYROGEN ADSORPTIVE LIGAND

Step 1:

D.I. Water	230 ml
+120 CSF Refined Cellulose	5 g
Vinyl imidazole	12.5 ml 85°C, 1 hour
Glycidyl Methacrylate (GMA)	2.5 ml
Ammonium persulfate (APS	0.5 g in 10 ml H_2O
Sodium thiosulfate (STS)	0.5 g in 10 ml H_2O
(LAE)	0.2 g

Step 2:

Link polyionene to the tertiary amine end groups - see Figure 13.

Procedure:

(a) Same procedure was followed for step 1 as in Examples 1-3 for grafting imidazole moities on solid surfaces.

(b) Referring to Figure 13:

Component A: 0.07 moles or 12.06 g tertiary diamine.

Component B: 0.07 moles or 12.25 g dichloro hexane.

The two components were added at the end of Step 1 reaction for overnight at 90°C. Nitrogen gas was not needed in this step. The product was washed twice and dried.

See Figure 11.

(c) Use

The above-prepared media was mixed in different amounts in 50 ml of 0.1 M sodium phosphate buffer, pH 6.6, doped with 2.0 microgram/ml of E.coli pyrogen agitated at room temperature for 1 hour. The results are shown in Table 4.

TABLE 4

PYROGEN REMOVAL FOR VARYING AMOUNTS OF MEDIA

Type of Media	Amount of Media applied (mg)	Amount of pyrogen bound to the media (micrograms)	% Pyrogen Removal
Media of Step 1	20	93.44	93.4
	100	97.32	97.3
	200	98.21	98.2
Media of Steps 1 and 2	20	98.49	98.5
	100	99.64	99.6
	200	99.68	99.7

Total amount pyrogen applied is 2.0 micrograms/ml x 50 ml. = 100 micrograms

EXAMPLE 5

INTRODUCTION OF QUATERNARY GROUPS TO THE HYDROPHOBIC SPACER ARM AS PYROGEN ADSORPTION LIGAND

This example shows a method of coupling a strong cationic group, such as a quaternary amine to the hydrophobic arm which already carries a weakly charged group, such as an amine prepared by Example 1.

The reaction is performed according to the following mechanism (See Figure 14):

Procedure

(a) An amount of matrix carrying diamino alkyl ligands prepared according to Example 1 was dispersed in DI water.

(b) A bifunctional reagent capable of reacting with amino groups existing on the matrix and also carrying pyrogen adsorptive groups was added to the dispersed media. An example of such a bifunctional reagent is glycidyl trimethyl ammonium chloride (Aldrich Chemical).

(c) The temperature of the mixture was raised to 90°C and reacted for more than 3 hours.

(d) The reaction mixture was "felted out" on a vacuum felting box and rinsed two to three times until the rinse water showed no change in pH and color.

(e) The media was dried.

EXAMPLE 6

COUPLING OF POLYMYXIN B AS PYROGEN ADSORPTIVE LIGAND

Polymixin B, (Sigma Chemical Corp.) a polyionene was coupled to the matrix of Example 1.

Procedure

The media was prepared as in Example 1 (decylamine) with 30 mls of water, equilibrated with 50 mls 0.1 M borate pH 8.2, 25 mg Polymyxin B in 5 mls borate buffer for 3.5 hours was added along with 5 mls borate buffer, 15 mg $NaBH_4$ for thirty minutes. The mixture was then washed with borate buffer after 5 hours of recirculation (last 1.5 hours with $NaBH_4$).

The mixture was then deactivated with 10 mls 1% glycine ethyl ester and 20 mg $NaBH_4$ overnight. 15 mg $NaBH_4$ was then added twice. The media was then washed with 30 mls borate buffer (pH 8.2) followed by 50 mls Gly-HCl (pH 2.3) and then 50 mls 0.1 M NaP + NaP + 0.25 M NaCl (pH 6.6).

Use

The media was then tested as in Examples 1 and 2.

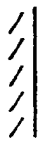
Experiment No.	Type of Media	Pyrogen Applied	Pyrogen left in solution	Amount of pyrogen removed
6a	GMA grafted	5 μg/ml of 200 ml total	4.63 μg/ml	75 μg
6b	GMA coupled with Polymyxin B		2.81 μg/ml	439 μg
6c	Media prepared in Example 1		3.21 μg/ml	359 μg

6d	6c coupled with Polymyxin B	0.18 μg/ml	965 μg

EXAMPLE 7

INTRODUCTION OF A POLYPEPTIDE AS A SPACER TO ENHANCE THE ADSORPTIVE FORCE OF THE LIGANDS TOWARD PYROGEN

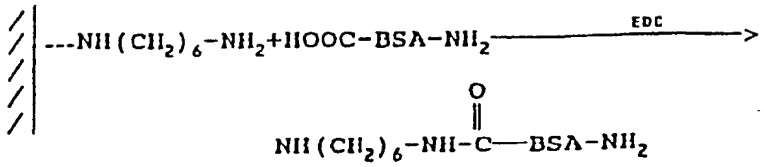
A polypeptide, such as polylysine (other protein such as albumin may also be used) was coupled to the carrier matrix as spacer to link the hydrophobic and charge functional groups to the matrix. An example:



Procedure

BSA Coupling to HDA cellulose pulp through carboxyl group of protein using EDC chemistry:

100 g wt. HDA-pulp (21% dry) was dispersed in 2.4 liters of 0.1 M NaCl containing BSA (10mg/ml). The final pH=4.5-4.7 was adjusted with dilute HCl or dilute NaOH. After 30 minutes agitation at room temperature 5.0 g of EDC was added in five portions 30 minutes apart. A pH of 4.5-4.7 was maintained with the addition of dilute acid or alkali. The next day the pH was raised to 8.2 with 6 N NaOH and the agitation continued for 3 hours. The BSA coupled pulp was washed with 1 M NaCl pH=4.0 adjusted with 1 M NaP (monobase), 1 M NaCl pH=8.6 adjusted with 0.5 M NaP (dibase) and finally with DI water. The total washing volume was 8 liters. Amount of BSA coupled was 169.95 mg/gm of matrix.



EDC is a coupling agent which activates the carboxyl groups in BSA to react with the amino groups on the matrix. EDC was purchased from Pierce Chemical and has the structure of 1-ethyl-3-(3 dimethyl amino propyl) carbodimide hydrochloride.

Results

The media was felted into 6-inch pads and dried at room temperature overnight. The next day it was dried at 60°C for 45 minutes. 0.5 gram media were made by packing in a 16 mm plastic tube as a mini column. The column was washed with 100 mls/device of 0.05 M NaP pH 7.15 400 mls of buffer + 200 ng/ml pyrogen/device at 3.0 mls/min.

Experiment No.	Matrix Material	Pyrogen Concentration Applied	Unbound	% Removal
1.	Matrix made in Example 1 (control)	200 ng/ml	24.3 ng/ml	87.9
2.	BSA coupled to #1	200 ng/ml	5.0 ng/ml	97.5
3.	BSA coupled to #1, followed by reacting with glycidyl trimethyl ammonium chloride as Example 4.	200 ng/ml	28.3 ng/ml	85.9

WHAT IS CLAIMED:

1. A filter media comprising a water insoluble carrier modified by a modifying polymer having a polymer chain and having along the polymer chain a pendent cationic substituent and a pendent hydrophobic substituent.

2. The media of Claim 1, wherein the cationic substituent is selected from the group consisting of primary, secondary, tertiary and quaternary amino groups.

3. The media of claim 1, wherein the hydrophobic substituent is a C_4 to C_{20} alkyl or aromatic substituent.

4. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of:

(a) a compound of the formula:

(i) $R^1\ R^2\ N{-}{-}X{-}{-}N\ R^3R^4$, or

(ii) $R^1\ R^2\ N{-}{-}X{-}{-}N\ R^3{-}{-}Y{-}{-}N\ R^4R^5$

wherein X and Y are each, independently, an aliphatic or aromatic substituent of 4 to 20 carbon atoms, and

R^1, R^2, R^3, R^4 and R^5 are each, independently, a hydrogen or aliphatic substituent of 1 to 3 carbon atoms, and

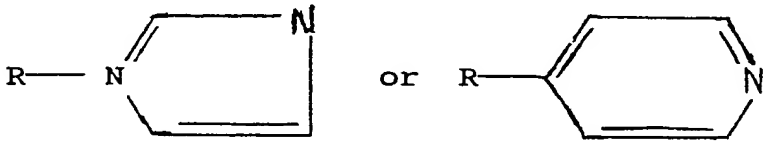
(b) a compound containing an epoxy group capable of direct coupling to an N on compound (a) and a vinyl group capable of bonding to the carrier.

5. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) a compound containing an epoxy group capable of direct covalent coupling to a substituent on the carrier and a vinyl group capable of free radical polymerization; and

(b) a compound having the formula:



wherein R is an alpha, beta-ethylenically unsaturated polymerizable radical capable of polymerization with the vinyl group of compound (a).

6. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) a compound of the formula:

$(NH_2\text{-}R^1)_n\ NH_2$ wherein R^1 is an aliphatic or aromatic substituent of 4 to 12 carbon atoms, and $_n$ is an integer

of 1 to 3, and

(b) glycidyl methacrylate.

7. The filter media of claim 6, wherein R is an aliphatic substituent of 6 to 10 carbon atoms, and $_n$ is 1.

8. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) diamino phenyl amino, and

(b) glycidyl methacrylate.

9. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) diamino diphenyl amino, and

(b) glycidyl methacrylate.

10. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) vinyl imidazole, and

(b) glycidyl methacrylate.

11. A filter media comprising:

a water insoluble carrier modified by a modifying polymer made from a polymerization of

(a) N (3-aminopropyl) methacrylamide, and

(b) glycidyl methacrylate.

12. The filter media of claim 1, further comprising a polyionene bonded to the polymer chain.

13. The media of Claim 4, wherein (b) is glycidyl methacrylate.

14. The media of Claim 1, wherein the carrier is cellulose.

15. The media of Claim 1, comprising a filter sheet of cellulosic fibrous filter elements and particulate filter elements.

16. The media of Claim 1, comprising filter elements of particulate immobilized in a porous matrix of cellulose fibers.

17. The media of Claim 16, wherein the cellulose fibers are highly purified alpha-cellulose.

18. The media of Claim 15, comprising at least 50% by weight of particulate filter elements.

19. The media of Claim 18, wherein the particulate filter elements are selected from the group consisting of diatomaceous earth, perlite and mixtures thereof.

20. A method of producing a modified filter media comprising applying to a water insoluble carrier a modifying polymer having a polymer chain and having along the polymer chain pendent cationic and pendent hydrophobic substituents.

21. The method of Claim 20, wherein the cationic substituent is selected from the group consisting of primary, secondary, tertiary and quaternary amino groups.

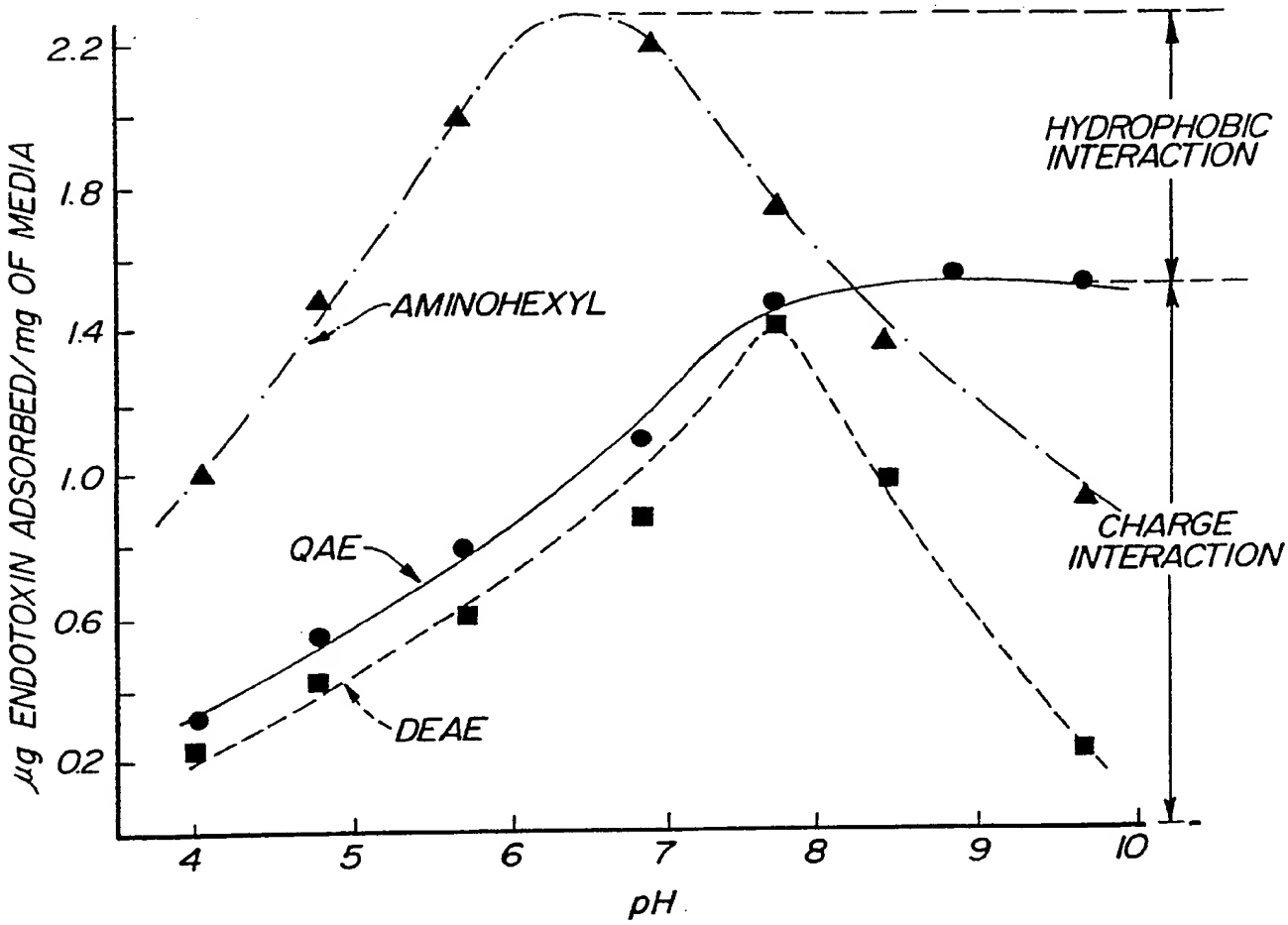
22. A method of producing the media of Claim 4, comprising reacting compound (a) with the carrier to produce an intermediate composition, followed by reacting compound (b) with the intermediate composition.

23. A method of removing pyrogen from an aqueous composition comprising contacting the solution with the media of Claim 1.

24. A method of removing pyrogen from an aqueous composition comprising passing the solution through the media of Claim 1.

25. The method of Claim 24, wherein the aqueous composition is a protein-containing composition.

FIG-1 THE EFFECT OF pH ON ENDOTOXIN ADSORPTION



Endotoxin Conc.: 40μg E-coli 026:B6 endotoxin dispersed in 20ml of 0.1M buffer.

Media Adsorption: 25mg media dispersed in above solution and agitated for one (1) hour.

Assay: Quantitative Chromogenic 1000 LAL of Whittaker Bio-Products.

FIG-2 EFFECT OF SALT ON PYROGEN REMOVAL

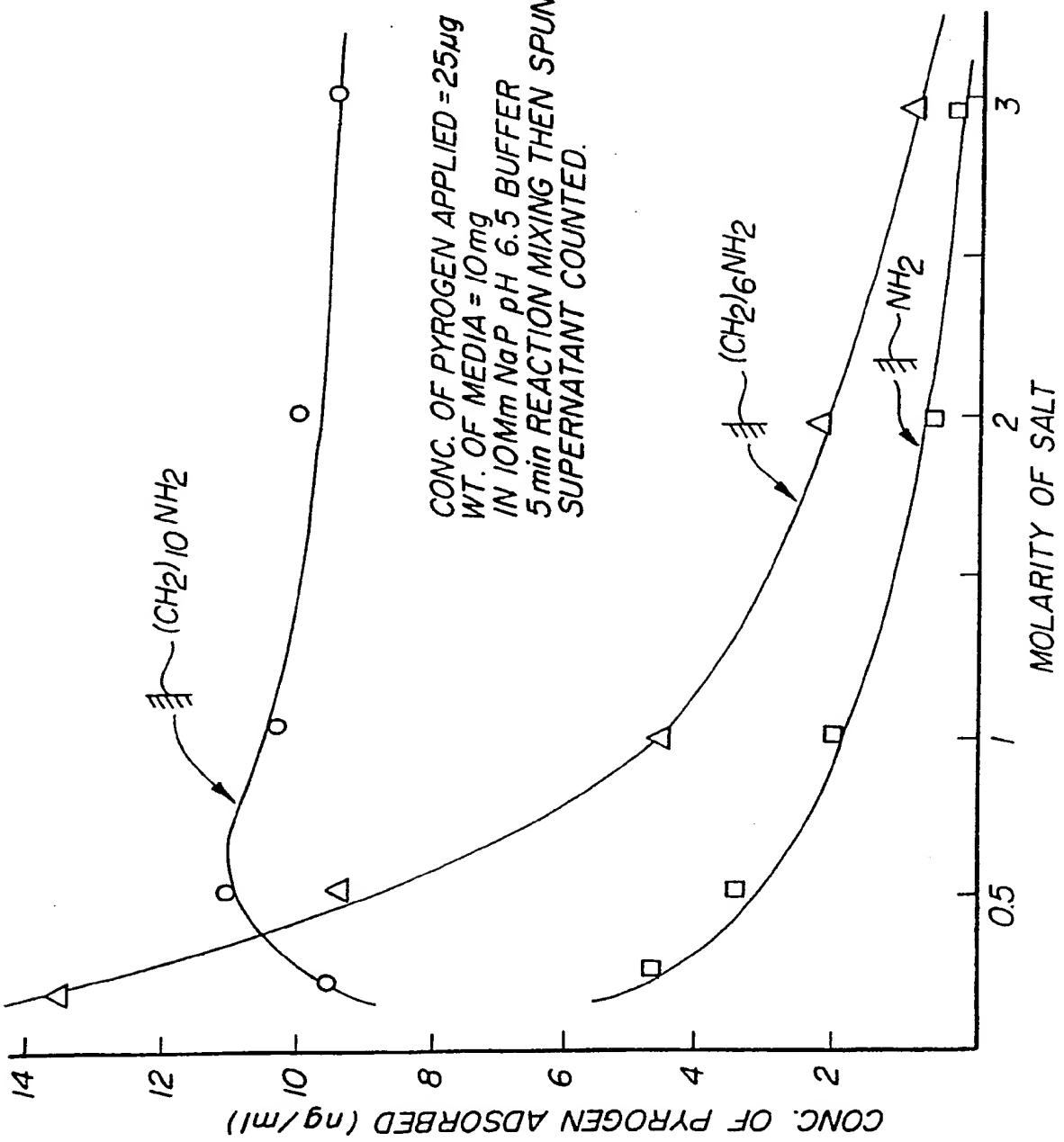
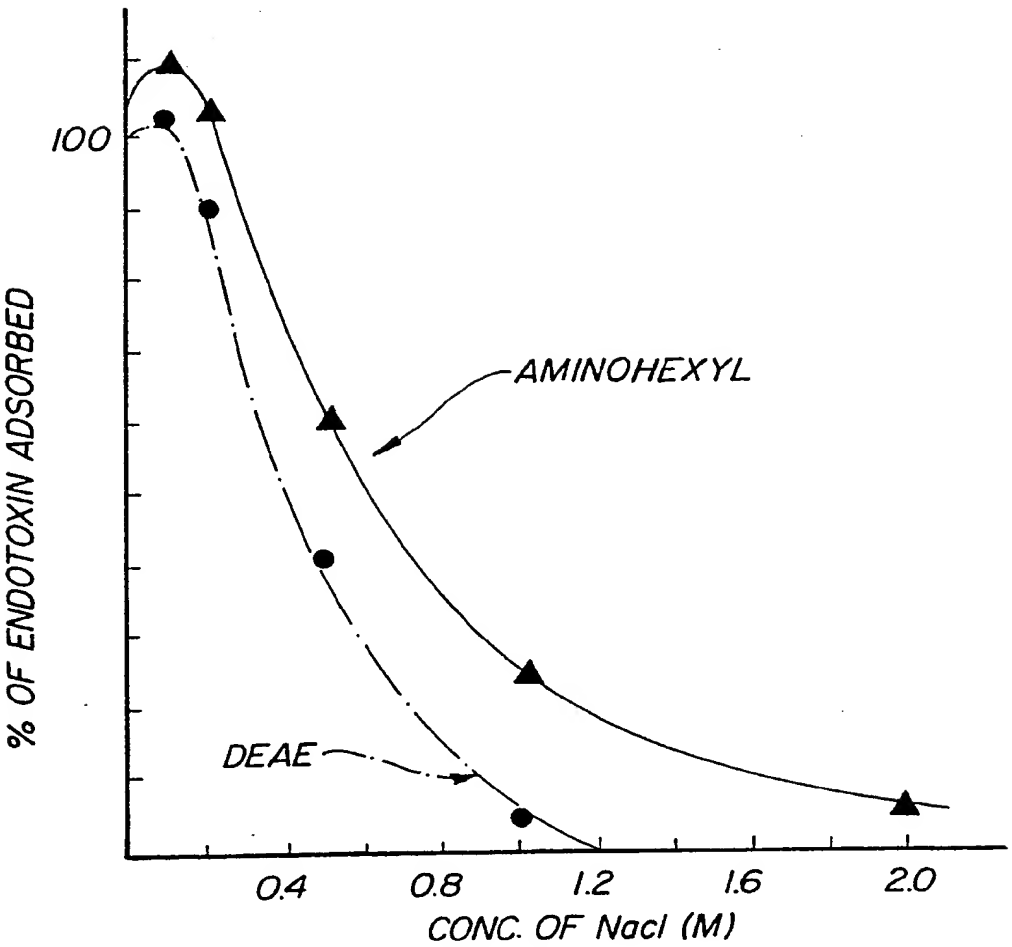


FIG-3 EFFECT OF SALT ON ENDOTOXIN ADSORPTION



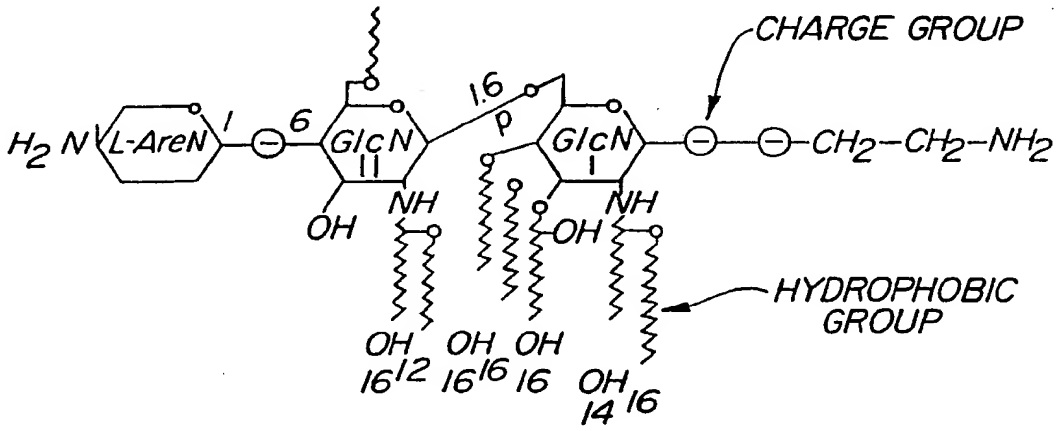
Endotoxin Conc.: 40mg E-coli 026:B_6 dispersed in 20ml of 50mM phosphate buffer at pH 7.0.

Matrix: 20mg added in above solution and shake for one (1) hour at r.t.

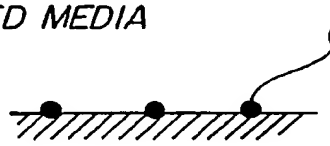
Assay: QCL-1000 from Whittaker Bio-Products.

FIG-4

a) PYROGEN STRUCTURE

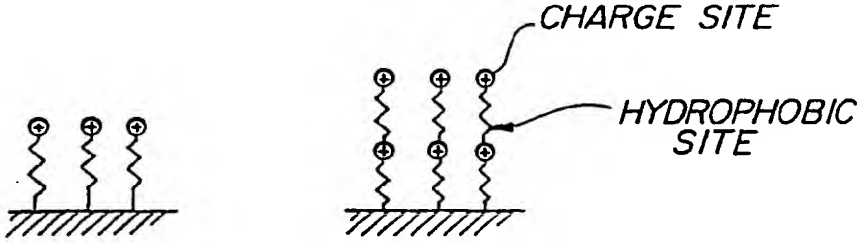


b) CHARGE MODIFIED MEDIA



Charge sites by coating are non-flexible and rely on depth for chance of catching pyrogens in a limited pH range.

c) INVENTION



Increasing charge density and strength as well as conformation flexibility through inserting a hydrophobic arm.

FIG-5 HYPOTHETICAL MECHANISM ON PYROGEN ADSORPTION BY THE FILTER CARRYING BOTH HYDROPHOBIC AND CHARGE INTERACTION FORCES.

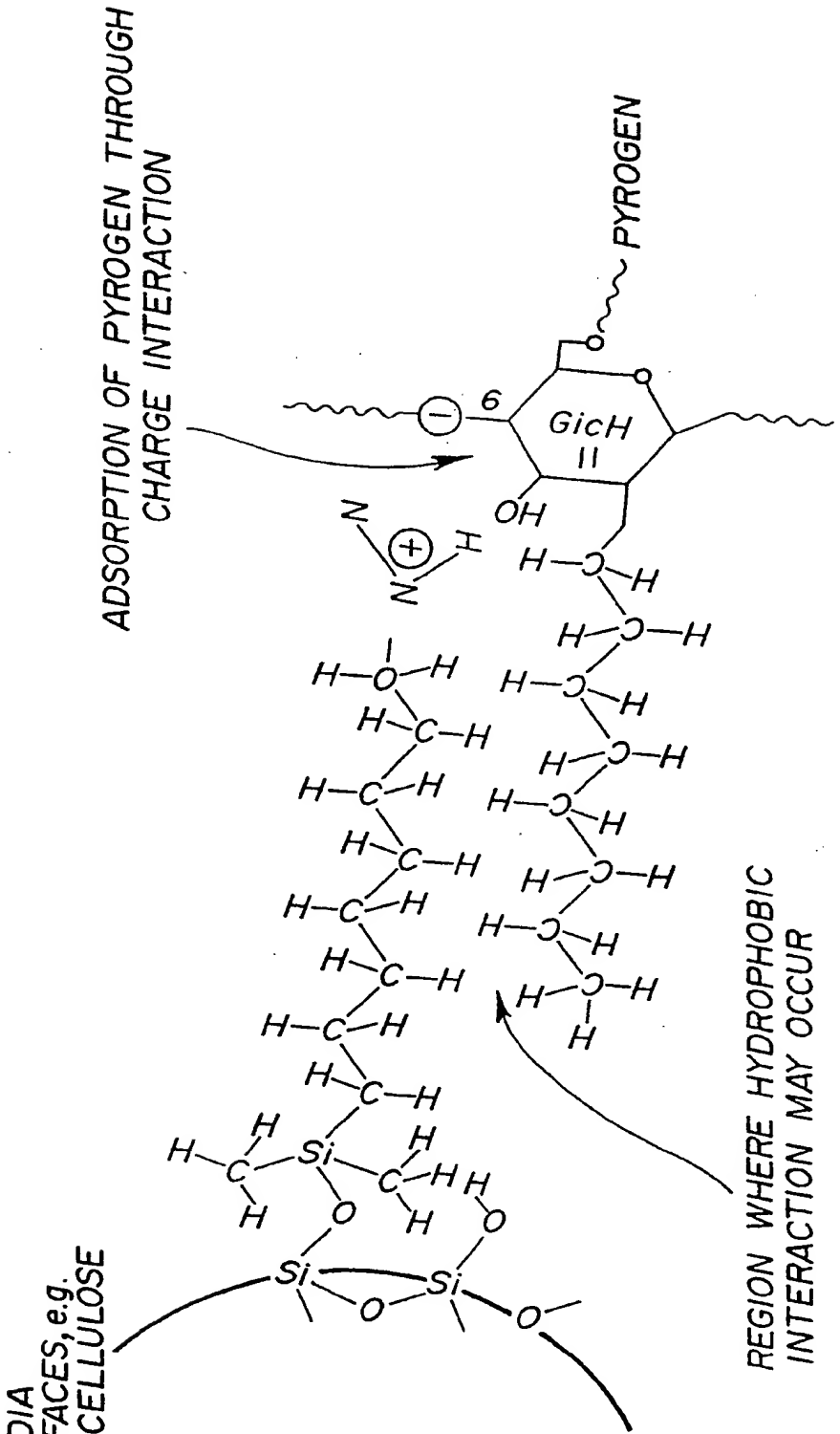
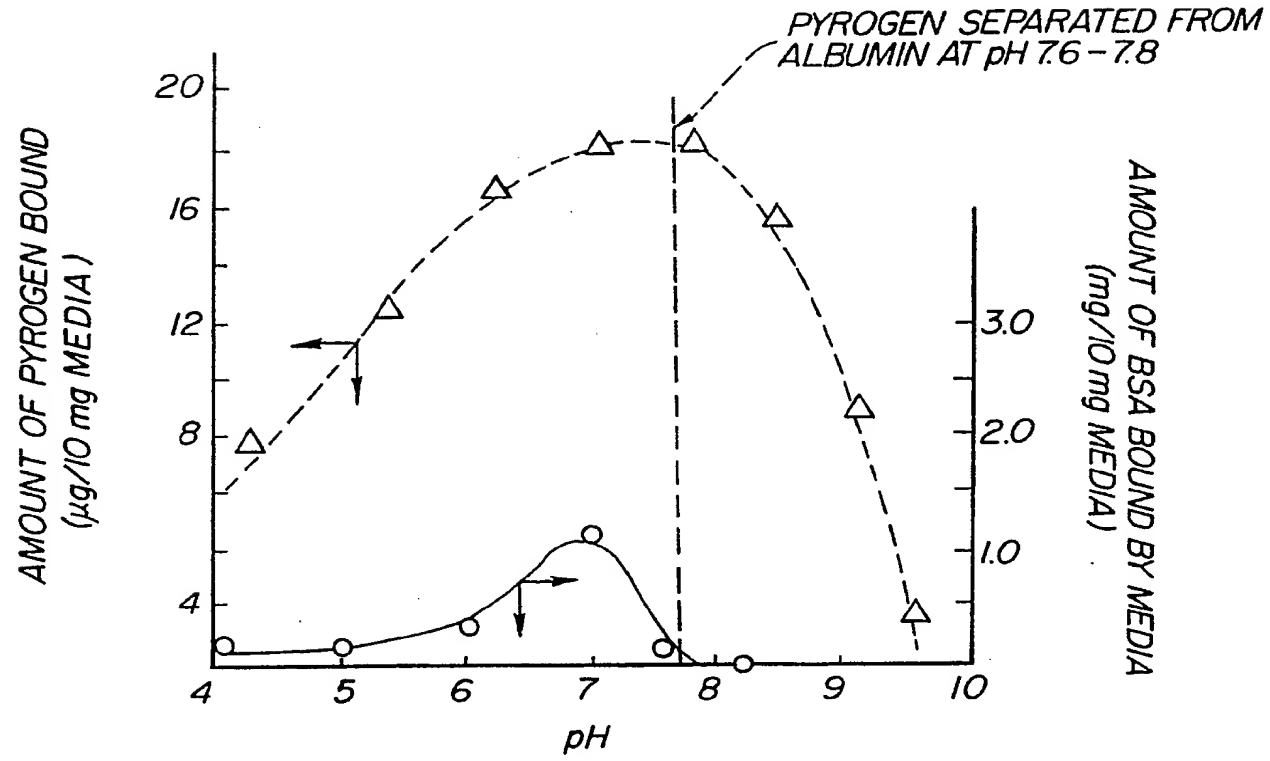


FIG-6 EFFECT OF pH ON PYROGEN REMOVAL FROM ALBUMIN

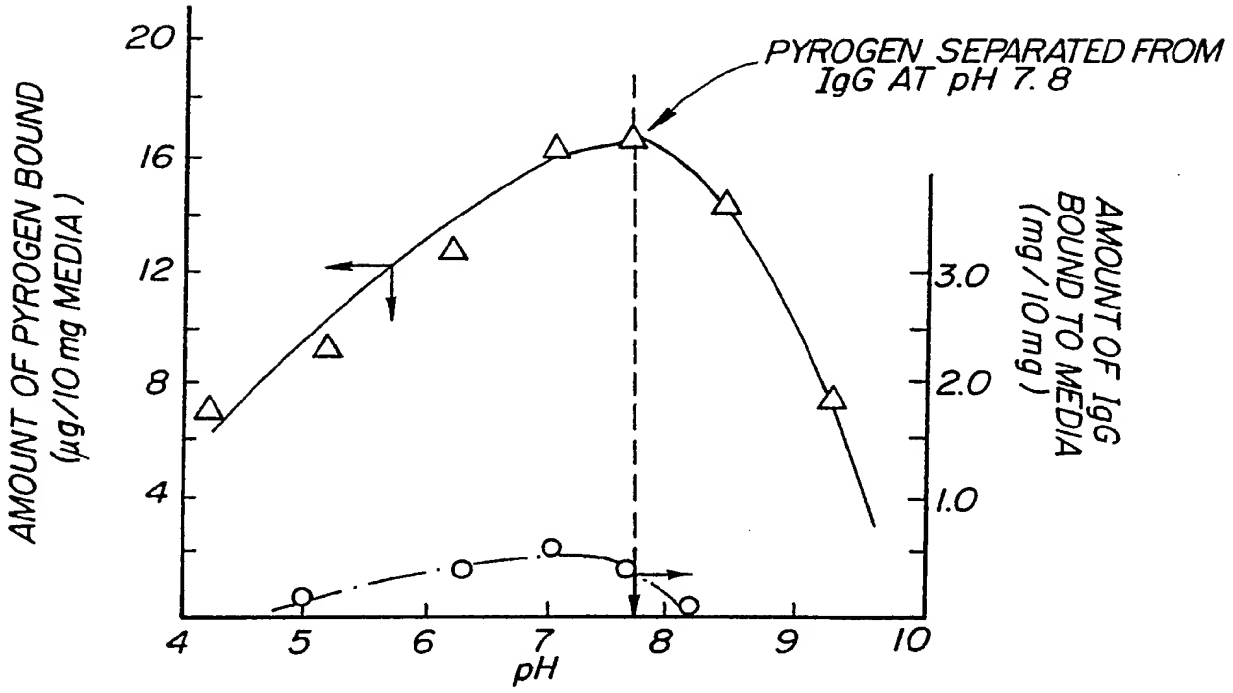


Solution: 20µg EL-coli pyrogen doped in 5mg BSA in 5ml 0.1 M buffer

Media: 10mg dispersed in test tubes

Testing condition: 1 hr. agitation and spin down the media

FIG-7 EFFECT OF pH ON PYROGEN REMOVAL FROM GAMMA GLOBULIN



Solution: 20µg E-coli pyrogen doped in 5 mg IgG in 5 ml 0.1 M buffer

Media: 10 mg dispersed in test tube

Condition: 1 hr. agitation and spin down the media

FIG-8 COUPLING OF DIAMINO ALKYL GROUPS THROUGH REACTING WITH EPOXY GROUPS IN GMA

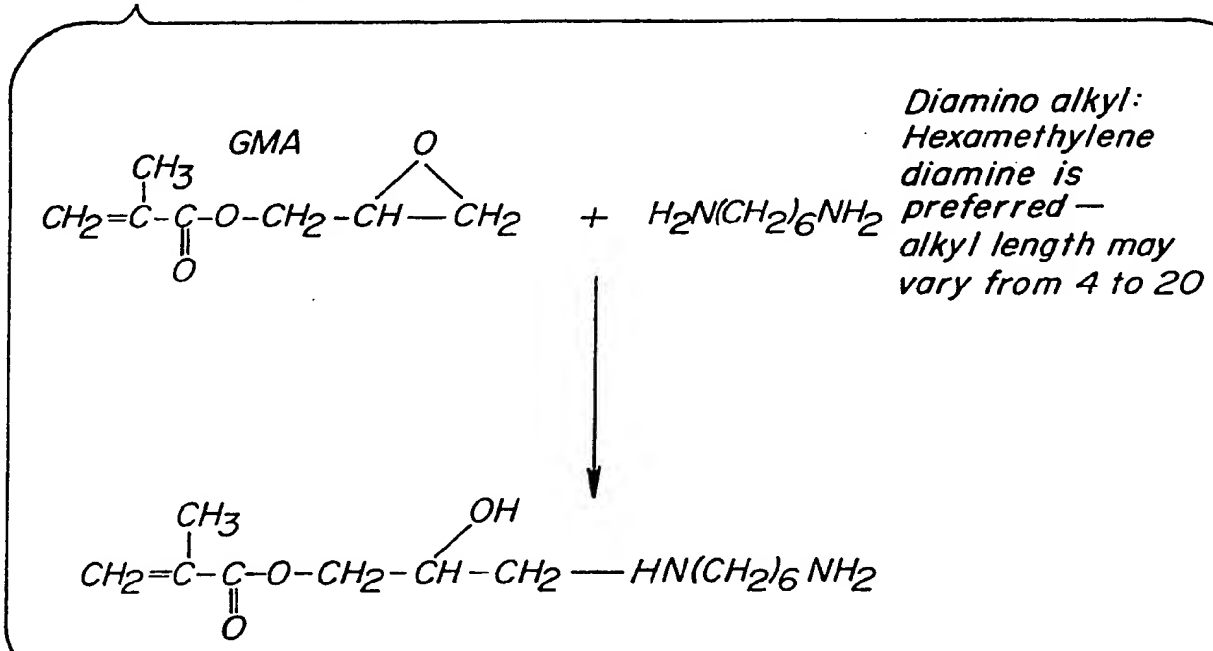


FIG-9 COUPLING OF AMINO ALKYL OLIGOMERS THROUGH GMA

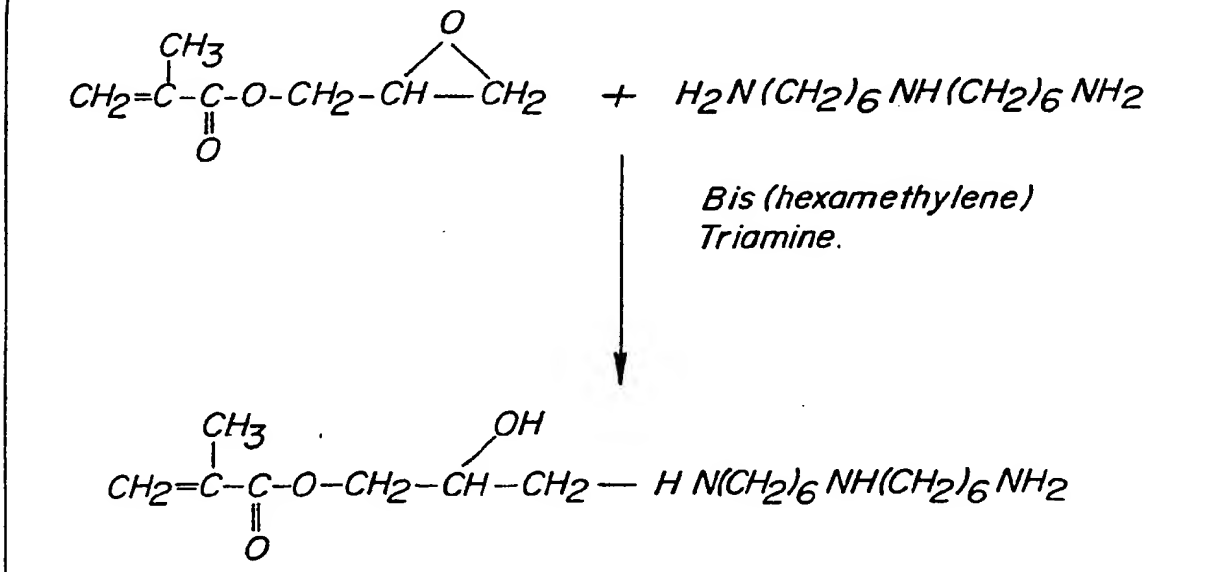


FIG-10 COUPLING OF AROMATIC DIAMINE GROUPS TO GMA

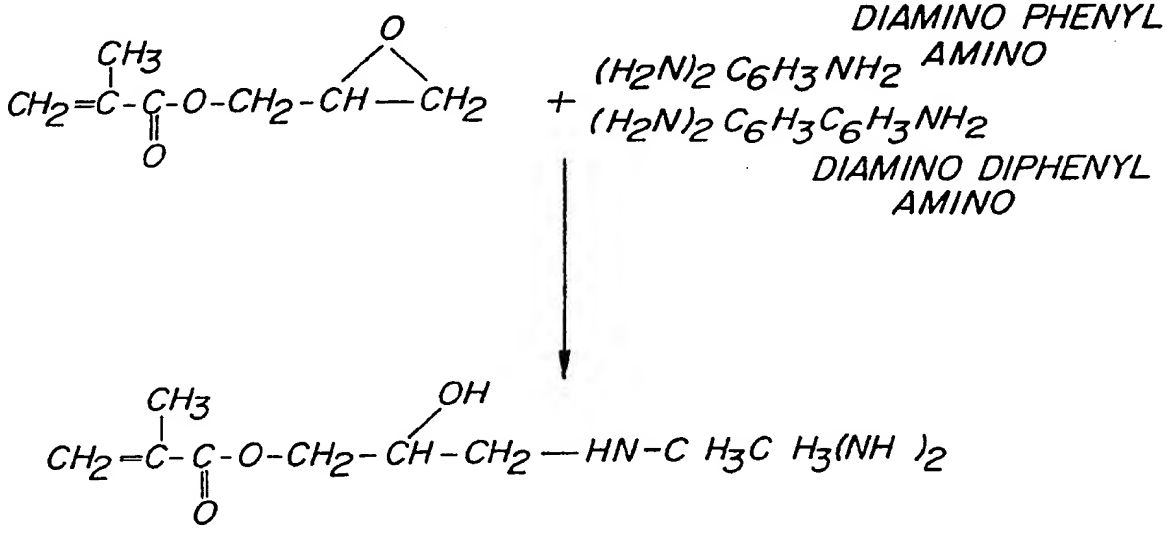


FIG-11 FORMATION OF COPOLYMERS CARRYING AROMATIC AMINO GROUPS

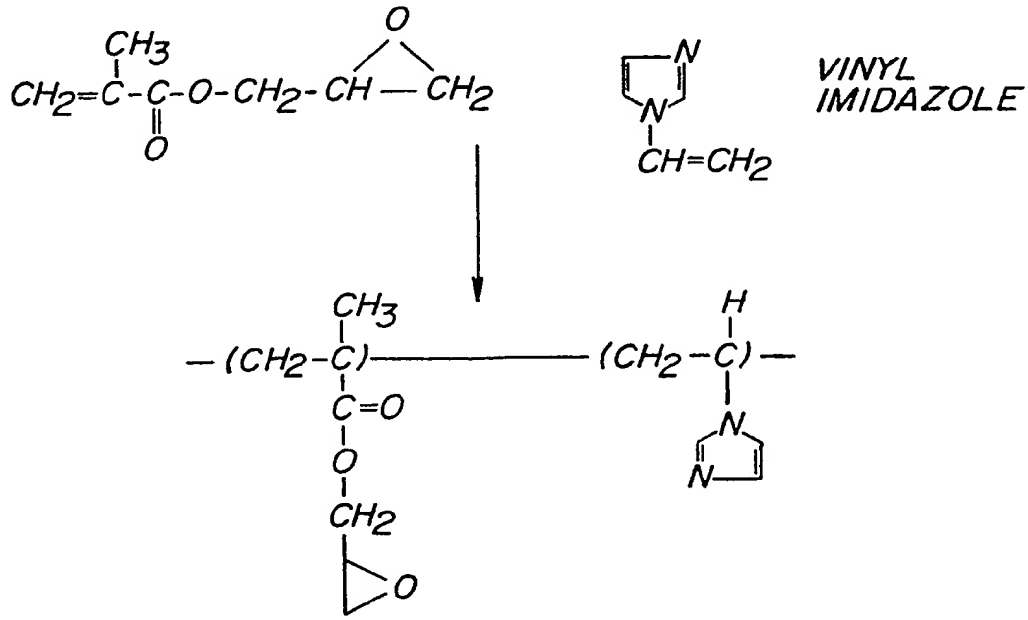
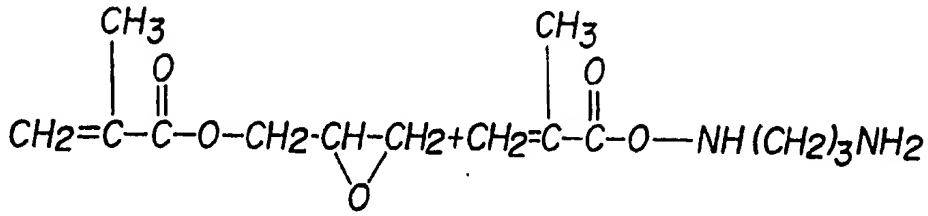


FIG-12

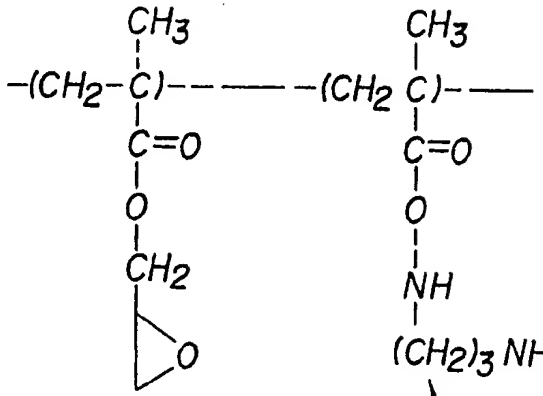
FORMATION OF COPOLYMERS WITH ACRYLIC MONOMERS CARRYING BOTH HYDROPHOBIC AND POSITIVE CHARGE AS FUNCTIONAL GROUPS.



GLYCIDYL METHACRYLATE

APMA
N (3 AMINOPROPYL METHACRYLAMIDE)

↓ POTASSIUM PERSULFATE
SODIUM THIOSULFATE



THIS ALKYL GROUP COULD BE VARIED FROM 3 TO 20.

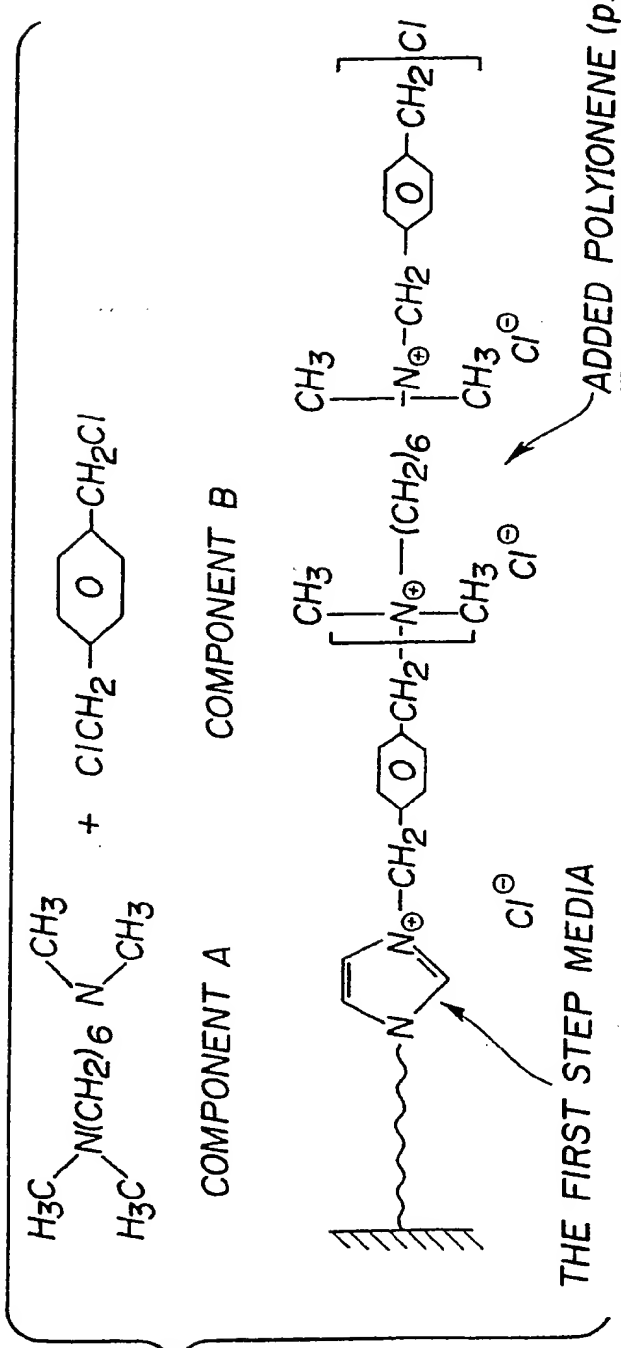

COMPONENT A
COMPONENT B
THE FIRST STEP MEDIA
ADDED POLYIONENE (pI)
TO ENHANCE THE IMIDAZOLE
FOR BETTER PYROGEN
ADSORPTION

FIG-13

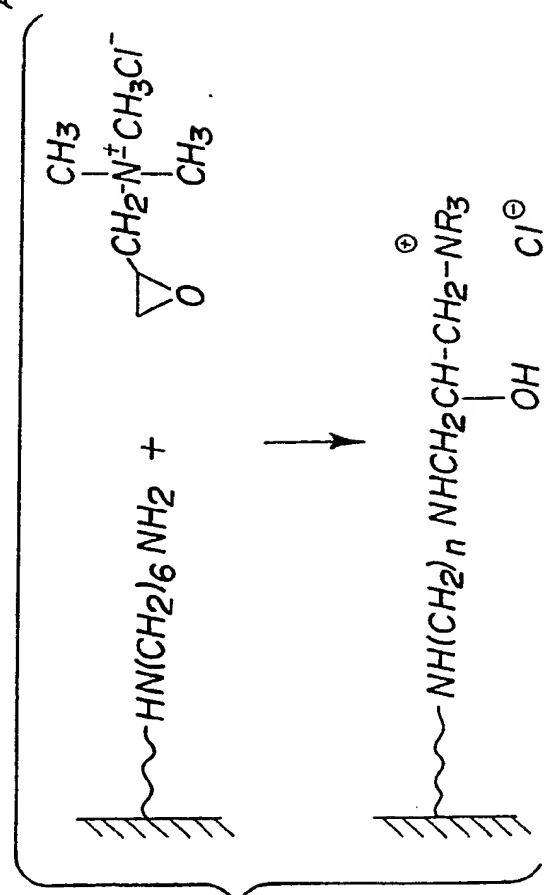


FIG-14

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05267

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): B01D 15/08
U.S.CL.: 210/679

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	210/500.29, 500.3, 500.31, 500.32, 500.43, 508 210/679, 691, 692 427/244

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,633,163 (HOU) 05 May 1987 See: the abstract; column 7, lines 35-54; column 9, lines 45-column 10, line 66; and column 15, lines 19-61.	1-25
Y	C.P. GERBA ET AL., "Endotoxin Removal by Charge-Modified Filters" Applied and Environmental Microbiology, Volume 50, No. 6, pages 1375-1377, published December 1985 by American Society for Microbiology. See the abstract and page 1377 left column, line 2-9.	23-25
A	J.P. NOLAN ET AL., "Endotoxin Binding by Changed and Unchanged Resins" Proceedings of the Society for Experimental Biology and Medicine, column 149, pages 766-770, published 1975 by the Society for Experimental Biology and Medicine, See the entire document.	1-25
	US, A, 4,381,239 (CHIBATA) 26 April 1983 See the entire document.	1-25
A	US, A, 4,511,473 (HOU) 16 April 1985 See the entire document.	1-25

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
30 OCTOBER 1990	30 JAN 1991
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	for W. GARY JONES

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	US, A, 4,411,795 (OLSON) 25 October 1983 See the entire document	1-25
A	US, A, 4,724,207 (HOU) 09 February 1988 See the entire document	1-25

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.